NREL-Amoco CRADA Phase 3

Bench Scale Report 1.7

Batch SSCF of Pretreated Corn Fiber with LNHST2

Project Title: Amoco-NREL **CRADA** with corn fiber

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Objectives

Several objectives were pursued in this **study:** (1) **Determine** if a solids loading of 20% corn fiber **has an** effect on the fermentation performance of the yeast strain LNHST2 compared to a 10% **solids** loading; (2) Ascertain the time it takes in batch mode to consume 90% of the soluble xylose; (3) Monitor the course of the soluble sugars, xylans, **and** glucans in the reactor **and** determine the ethanol **and** by-product **yields;** and ***(5)*** With these data, close the carbon balance around the **SSCF** for each solids loading.

Background

This experiment **is** designed to support the **Task 3 PDU** activities, which will examine the operability of the pilot plant **with** APR-pretreated corn fiber solids, **using** the organism LNHST2. Results **obtained** at the bench scale can then be compared to results obtained at the **PDU** to examine the **ability** to scale up lab data.

Materials and Methods

Inoculum

Inoculum **was** generated in YEPD (1% w/v yeast extract, 2% w/v peptone **and** 2% w/v glucose, pH 5.0) in two stages at 30°C **and** 150 rpm. The first stage, consisting of 50 mL of YEPD in a 250 mL baffled Erlenmeyer **flask,** was inoculated **with** a colony from **a YEPX** plate (1% w/v yeast extract, 2% w/v peptone **and 2%** w/v glucose, pH *5)* **and** incubated for 14.5 hours. **A** 10% w/v inoculum was added to two ***500*** mL baffled Erlenmeyer **flasks** containing 130 mL of YEPD each. The **flasks** were incubated for only 6 hours; by that time, a majority of the glucose was consumed, but not all (exponential growth stage). The contents of **both flasks were** combined, mixed **and split** to ensure the same **inoculum** for each fermentor.

***Enzyme** and nutrients*

Cellulase enzyme was used with an activity determined at NREL to be 80 **IFPU/mL.** Based on this activity and **an** assumed 25% cellulose content in **raw** corn fiber, **an** amount of cellulase **was** added to each fermentor corresponding to 10 IFPU/g cellulose. In addition to cellulase, glucoamylase was acquired from Enzyme Development Corporation with a reported activity of 285 IU/mL. The enzyme was added at a level of 2 IU/g of starch based on a 20% starch content assumption in raw corn fiber. Each enzyme preparation was filter sterilized through 0.2 µm filter before being added to the fermentors.

Corn Steep Liquor (CSL) (1% w/v) was added as a nutrient source to each fermentor. To prepare the solution, the pH of a 50% w/w dilution of the purchased CSL was first adjusted to 5.0 with sodium hydroxide pellets and autoclaved

Corn fiber

Corn fiber (CF) was obtained from the APR. As the pH of the CF is very low, the pH needs to be adjusted to 5.0 for the fermentation. In order to do this, 1500 grams of material was weighed out, 210 mL of water was added to facilitate mixing, and 49 mL of a 10M NaOH solution was added to bring the pH to 5.0. Based on the added liquids, the percent solids was recalculated (25.59%) and verified by measuring the solids level with an IR balance (25.50%).

Fermentations

The amount of CF necessary to give 10% and 20% solids in a 1.2-L final volume was weighed and placed into the two BioFlo III fermentors with a known amount of additional water and autoclaved for one hour. Each fermentor was inoculated with the appropriate amount of inoculum, CSL, enzymes, and enough water to bring the solids level to the appropriate level. Each fermentor was operated at 30°C, 150 rpm, and pH 5.0, which was controlled with the addition of 3 M sodium hydroxide.

Sampling and Analysis

Initial and final samples were obtained and a total compositional analysis was performed on the solid and liquid fractions. Samples were taken every 24 hours and the liquid fraction was analyzed for cellobiose, apparent xylitol, lactic acid, glycerol, acetic acid, HMF, and furfural, as well as monomeric and oligomeric sugars (glucose, xylose, galactose, arabinose, and mannose). Colony forming units (CFUs) were also performed on each sample to monitor the cell population.

Results and Discussions

Effect of Solids Loading on Fermentation Performance

Based on glucose and xylose utilization, ethanol production, and cell counts, there is no discernible effect from the high solids loading (20% extruded corn fiber) on the fermentation performance of LNHST2. The initial monomeric and soluble glucose concentrations in the 20% solids SSCF are twice those of the 10% solids SSCF, as seen in Table 1. Within 22 hours of fermentation, 86-90% of the glucose was consumed in both the 10% and 20% SSCF. By normalizing the glucose results, it is apparent that a similar percentage of glucose is consumed regardless of the solids loading (Table 1).

Table 1: Soluble glucose utilization in 10% and 20% corn fiber solids **SSCF**

Elapsed Time (h)	**20% solids** Soluble Glucose	**10% Solids** Soluble Glucose (g/L)	**20% solids** Normalized Glucose (%)	**10% Solids** Normalized Glucose (%)
0	57.84	30.13	100.0	100.0
22	8.10	3.02	14.0	10.0
46	***5.69***	2.55	9.84	8.46
68.5	5.45	2.38	9.42	7.90
91.5	5.36	2.52	9.27	8.37
120	5.37	23 1	9.28	7.67

Table 2 and Figures 1 and 2 show the soluble xylose (monomeric and oligomeric xylose) concentrations. In contrast to glucose, xylose consumption in the two reactors **is** not exactly the same. It is not known to what extent analytical errors **are** responsible for the inconsistency, but it should **be** noted that the **initial** xylose concentration levels are quite low, making the analysis less accurate. The normalized soluble xylose values show **a** slight increase in the xylose consumption **at** the 10% solids level over the 20% solids level (Table 2). Interestingly, **an** increase in oligomeric xylose is observed **between** zero **and** 22 hours at **both** solids levels (Figures 1 and 2). This may be due to **an** analytical error on the time zero sample, **since** there is no evidence of xylanase activity within the cellulase enzyme complex. Beyond the first 22 hours of the SSCF, the oligomeric xylose concentration remained unchanged

Unfortunately, 90% xylose consumption **(a PDU** objective) was not achieved within the duration **of** the experiment (120 h). Only 64.6% of the monomeric xylose **was** consumed in the **20%** SSCF **and** 85.1% **was** consumed in the 10% SSCF in 120 h. Based on the utilization **of** monomeric xylose, the consumption rate at the 20% level **was** 0.041 g/L-h, only slightly lower than the 0.053 g/L-h at 10% solids.

Table 2: Soluble xylose utilization in 10% and 20% corn fiber solids SSCF

	20% solids		**10% Solids**		**20% solids**	**10% Solids**
Elapsed Tune (h)	Total Soluble Xylose (g/L)	Monomeric Xylose (g/L)	Total Soluble Xylose (g/L)	Monomeric Xylose (g/L)	Normalized Monomeric Xylose	Normalized Monomeric Xylose (%)
0	18.00	3.93	10.39	2.08	100	100
22	25.83	3.24	11.73	.9 1	82.4	43.8
46	24.80	2.05	11.34	.5	52.2	24.0
68.5	23.89	1.56	11.23	.37	39.7	17.8
91.5	24.27	1.39	11.31	.3 1	**35.4**	14.9
120	24.81	1.39	10.96	.3 1	35.4	14.9

Another parameter that can be examined to determine if the solids loading has an effect on the fermentation performance is ethanol production. Unfortunately, problems were encountered with the analysis of the initial solids samples. Without this piece of data, theoretical ethanol yields cannot be calculated. Nevertheless, ethanol yields were calculated based on the initial solids loadings and expressed as grams of ethanol produced per gram of initial solids. The data show that there was no substantial difference in the ethanol production at 20% solids compared to 10% solids (Table 3). The yield profile was similar in the two reactors. In both vessels, the ethanol concentration reached its peak value within approximately 70 hours of SSCF.

Table 3: Ethanol Production in 10% and 20% extruded corn fiber solids SSCF

Elapsed Time (h)	**20% solids** Residual Ethanol Concentration (g/L)	**10% Solids** Residual Ethanol Concentration (g/L)	**20% solids** Ethanol Yield (g ethanol/ g solids)	**10% Solids** Ethanol Yield (g ethanol/ g solids)
0	1.08	1.01		
22	32.07	16.69	0.148	0.157
46	34.57	17.61	0.167	0.166
68.5	38.46	18.50	0.187	0.175
91.5	37.85	18.70	0.184	0.177
120	37.05	18.54	0.180	0.175

In addition to sugar utilization and ethanol production, cell concentration was also monitored in both fermentations. The cell concentrations were similar in the two reactors: 9.5×10^7 cells/mL in the 20% solids and slightly greater at 1.4×10^8 cells/mL in the 10% solids after 22 hours of growth. A decline in cell growth was observed at both solids levels beyond 70 hours of SSCF, perhaps as a result of starvation from monomeric sugars.

***Fermentation** Duration*

Within 22 hours, **a majority** of the soluble glucose is consumed and a **majority** of the ethanol is produced at both solids levels (Figure 3). Only a small amount of the monomeric xylose and none of the oligomeric xylose is utilized within that period. Over the rest of the fermentation, more monomeric xylose is converted to ethanol (Figure 3).

Yields, By-products, and Sugar Conversions

Yields and by-products were calculated based on the total glucose and xylose present at each solids loading. Glucan (in equivalent glucose) conversion in the 10% solids SSCF was 87.64% compared to 84.37% at 20% solids (*see* Table 4). This low conversion is due to the inefficient conversion of cellulose and starch to glucose during pretreatment. This is also evidenced in the xylose conversions where only 15.86% and 14.23% of the total available xylan (in equivalent xylose) is converted in the 10% and 20% solids SSCFs respectively, The lack of monomeric xylose production during pretreatment is even more severe than glucose as the enzyme preparation does not work effectively against oligomeric xylose and xylose in the solids.

Table 4: Sugar Conversions and Ethanol Yields based on Total Glucose and Xylose

Initial Solids Loading	10%	20%
Glucose Conversion (%)	**87.64**	**84.37**
Xylose Conversion (%)	15.86	14.23
Ethanol Process Yield (% theoretical)	60.13	50.61
Ethanol Metabolic Yield (% theoretical)	91.52	82.63

The ethanol process yield (based on total glucose and xylose) at 10% solids was 60.13% of theoretical **and** lower at 50.61% of theoretical at 20% solids (see Table **4).** The ethanol metabolic yield at 10% solids was also higher (91.52%) than at **20%** solids (82.63%). This result could indicate **that** there is an effect of the solids loading on the fermentation performance. However, when the carbon balance is examined (see Table ***5),*** the closure at the 10% solids level is well over 100% indicating that the obtained **yield** values could be inflated (analytical error). In addition, the 20% solids SSCF closure is under 100% indicating that the yields **at** the 20% solids loading could **be** deflated. One reason for the **disparity** in the carbon balance closures is **that** the value **used** to calculate the glucose concentration in the solids **is** very sensitive to the percent insoluble solids, which **is** calculated and not determined.

The major by-products observed were glycerol at 0.025 g/g consumed sugar and 0.029 g/g consumed sugar and xylitol at 0.052 g/g consumed sugar **and** 0.025 g/g consumed sugar in the 10% and **20%** solids SSCF respectively (see Table ***5).***

Table *5:* Product Distribution for the 10% and 20% Solids SSCFs (expressed **as grams** of product per 100 grams of consumed glucose and xylose)

Product	10% solids Loading	20% solids Loading
Ethanol	46.77	42.22
Carbon Dioxide	47.29	41.55
Cell Mass	5.02	5.08
Glycerol	**2.54**	2.91
Acetic Acid	0.26	0.93
Succinic Acid	**1.64**	0.38
Xylitol	5.02	2.50
Total	**108.54**	95.58

Conclusions

The time it took for the majority of the monomeric glucose to be consumed **was** just 22 hours. Xylose **was** taken up at **a** slower rate. Based on the available data, there **is** no discernible effect of the solids loading on the fermentation performance of LNHST2. The **cell** concentration in the 20% solids fermentation was similar to **that** in **the** 10% solids fermentation, **and** 3.8% w/v ethanol was produced **from** the 20% solids, double the 1.9% produced from the 10% solids. The 3.8% w/v ethanol and 1.9% w/v ethanol produced in the 20% and 10% solids fermentations, respectively, exceed the theoretical yields based on the consumed soluble sugars. The **solids data** will **allow** us to calculate the true yield coefficients.

Another point of interest in **this** experiment is the **lack** of conversion of oligomeric xylose to monomeric xylose. In the absence of xylanase activity in the cellulase enzyme preparation, **it** seems clear **that** the pretreatment procedure needs to convert more **xylans** to monomeric **xylose** in order to capitalize on the xylose-fermenting capability of the **yeast strain** LNHST2.

The insoluble **solids** content **of** pretreated biomass **is** critical in calculating the glucose **and** xylose present in biomass. Without a reliable value **of** insoluble **solids,** the value of the yield information **is diminished.** We **should** strive in the **future** to **come** up with a better method for determining **this** value.

Figure 1: Soluble Sugar Profile in 20% Corn Fiber Solids SSCF with LNHST2

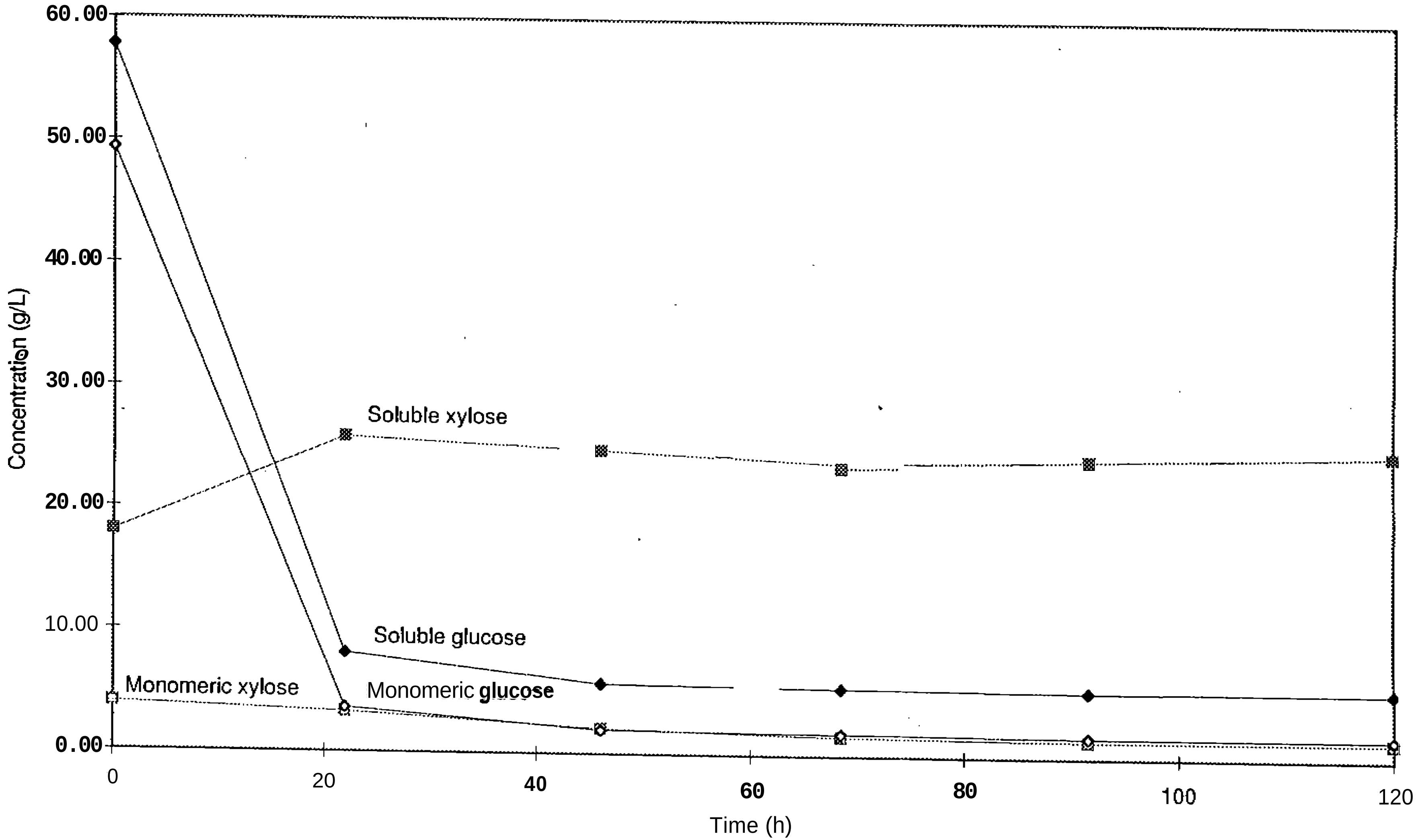


Figure 2: Soluble Sugar Profile in 10% Corn Fiber Solids SSCF with LNHST2

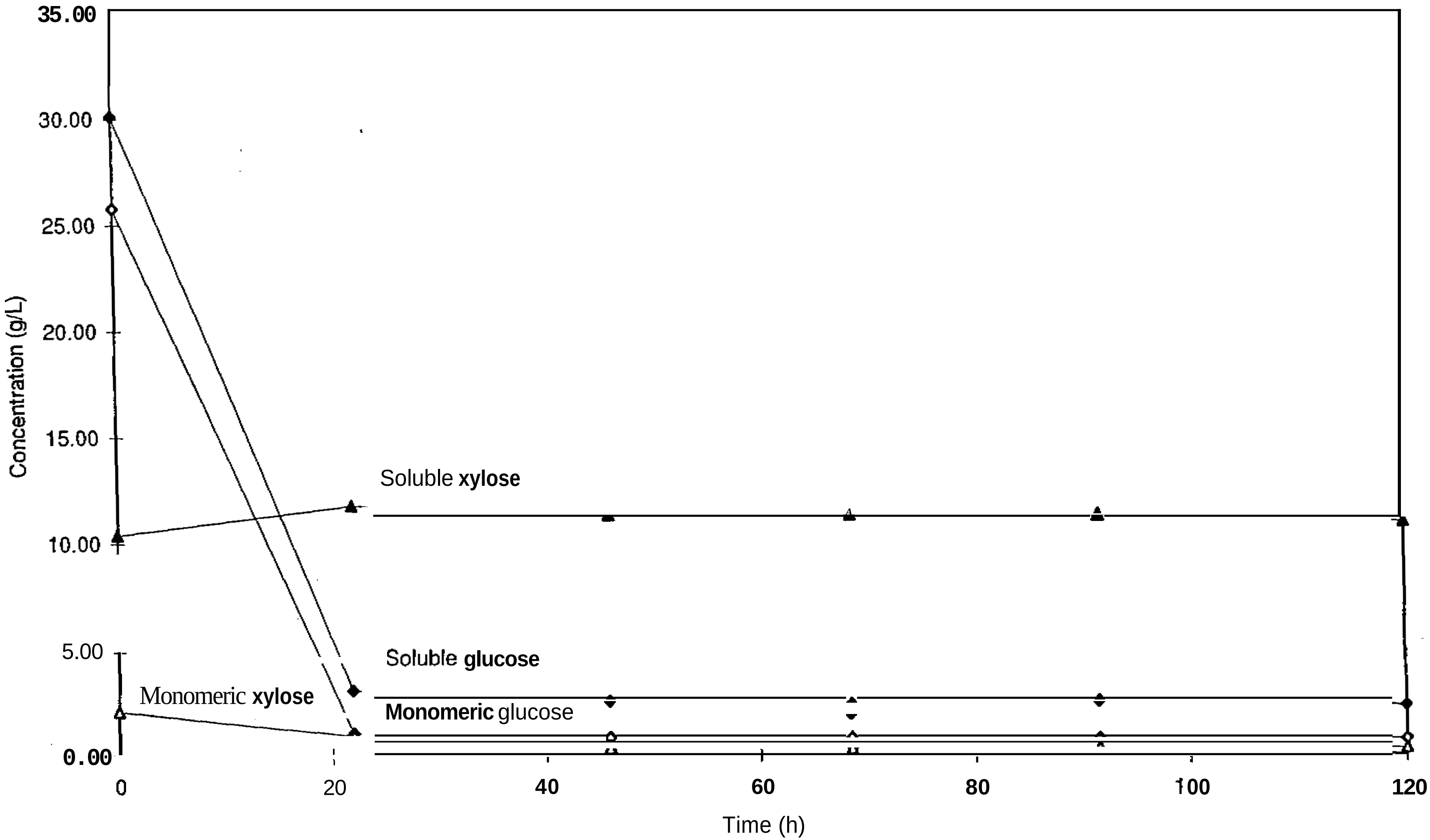
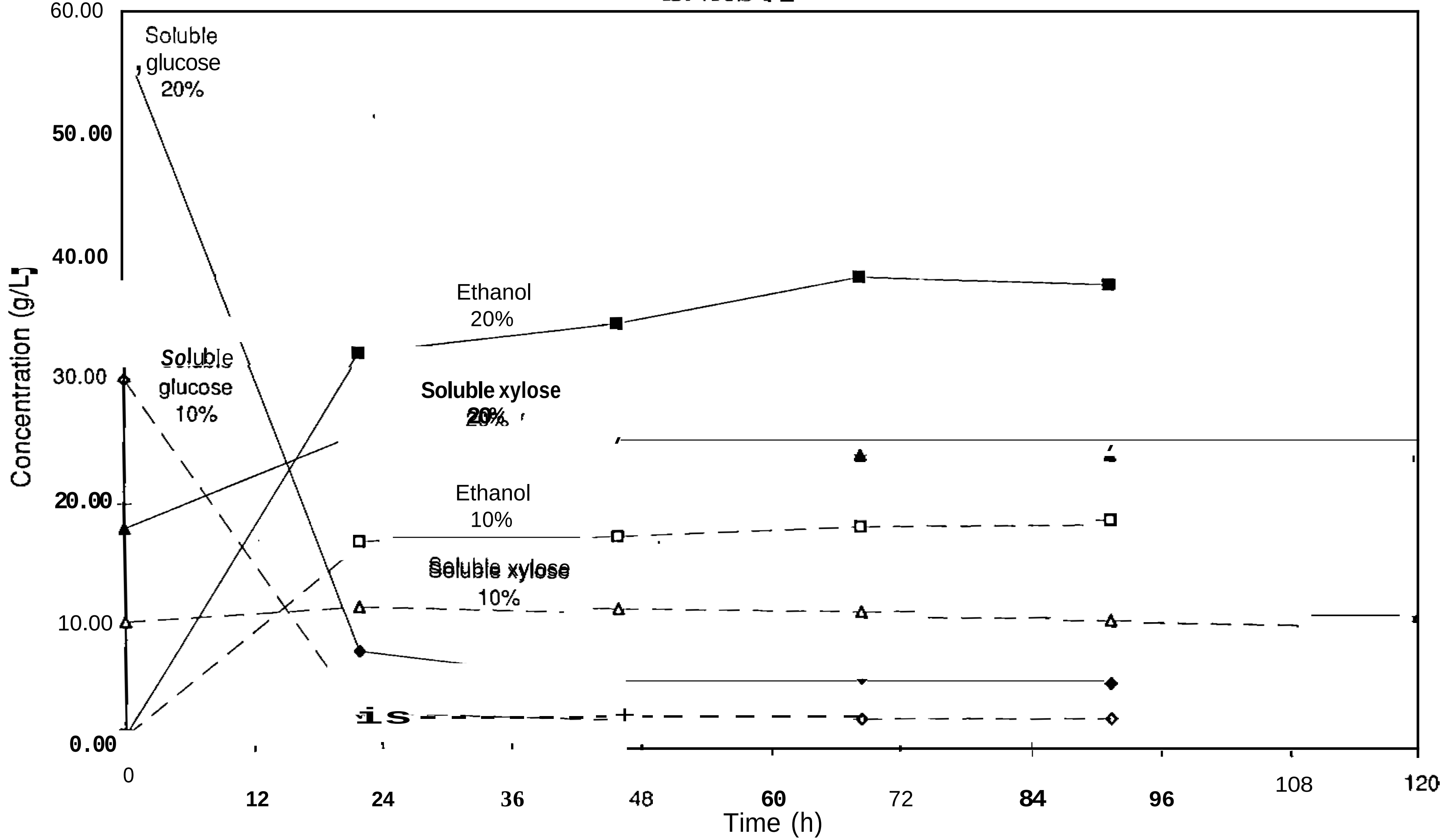


Figure 3: Comparison of 10% and 20% Corn Fiber Solids SSCF with LNHST2



Raw Data

20% Initial Solids Level - original CAT task data

		YSI	LC	YSI	LC	LC			Hauser				Hauser			Hauser	
Elapsed time (h)	Solids (%)	Glucose (g/L)	Glucose (g/L)	Ethanol (g/L)	Ethanol (g/L)	Xylose (g/L)	Cellobiose (g/L)	Monomeric Glucose (g/L)	Soluble Glucose[1] (g/L)	Oligomeric Glucose[1,2] (g/L)	Monomeric Xylose (g/L)	Soluble Xylose (g/L)	Oligomeric Xylose[2] (g/L)	Galaclose (g/L)	Total Galaclose (g/L)	Arabinose (g/L)	Total Arabinose (g/L)
0	19.40		49.65	0.97	1.08	7.44	5.25	49.32	57.84	8.52	3.93	18.00	14.07	1.05	4.20	12.18	20.22
22	15.78	0.36	0.90	34.10	32.07	5.92	0.00	3.60	8.10	4.50	3.24	25.83	22.59	0.84	5.01	12.00	21.42
46		0.19	0.00	37.20	34.57	3.33	0.00	1.94	5.69	3.75	2.05	24.80	22.75	0.87	5.17	11.90	20.52
68.5	15.20	0.19	0.00	37.10	38.46	2.93	0.00	1.80	5.45	3.65	1.56	23.89	22.33	0.85	4.93	11.61	19.66
91.5	15.29	0.16	0.00	38.29	37.85	2.83	0.00	1.69	5.36	3.67	1.39	24.27	22.88	0.89	5.05	11.36	19.95
120	14.91	0.16		37.05			0.00	1.69	5.37	3.68	1.39	24.81	23.42	0.96	5.21	11.36	20.00
Updated CAT task values																	
0							5.07	48.78	57.48	8.70	3.12	17.67	14.55	0.60	4.14	12.27	20.25
120							0.00	1.86	5.17	3.31	1.35	23.69	22.34	0.85	5.29	11.14	19.61

10% Initial Solids Level

		YSI	LC	YSI	LC	LC			Hauser							Hauser	
Elapsed time (h)	Solids (%)	Glucose (g/L)	Glucose (g/L)	Ethanol (g/L)	Ethanol (g/L)	Xylose (g/L)	Cellobiose (g/L)	Monomeric Glucose (g/L)	Soluble Glucose[1] (g/L)	Oligomeric Glucose[1,2] (g/L)	Monomeric Xylose (g/L)	Soluble Xylose (g/L)	Oligomeric Xylose[2] (g/L)	Galactose (g/L)	Total Galactose (g/L)	Arabinose (g/L)	Total Arabinose (g/L)
0	10.28		24.30	1.02	1.01	3.67	3.37	25.76	30.13	4.37	2.08	10.39	8.31	0.37	1.93	6.12	10.65
22	7.98	0.08	0.00	16.95	16.69	1.82	0.00	1.03	3.02	1.99	0.91	11.73	10.82	0.39	2.33	5.63	9.75
46		0.09	0.00	18.05	17.61	1.02	0.00	0.84	2.55	1.71	0.50	11.34	10.84	0.28	2.19	5.45	9.59
68.5	7.99	0.10	0.00	18.68	18.50	0.84	0.00	0.79	2.38	1.59	0.37	11.23	10.86	0.30	2.18	5.14	9.46
91.5		0.07	0.00	18.63	18.70	0.82	0.00	0.76	2.52	1.76	0.31	11.31	11.00	0.33	2.07	4.99	9.09
120	8.01	0.07		18.54			0.00	0.75	2.31	1.56	0.31	10.96	10.65	0.42	1.93	4.83	8.62
Updated CAT task values																	
0							3.37	25.76	29.39	3.63	2.08	9.62	7.54	0.37	2.08	6.12	t0.47
120							0.00	0.84	2.20	1.36	0.29	10.74	10.45	0.33	2.17	4.74	8.85

[1] Includes cellobiose

[2] Difference between soluble and monomeric values

Byproducts						
Mannose (g/L)	Total Mannose (g/L)	Succinic Acid (g/L)	Lactic Acid (g/L)	Glycerol (g/L)	Acetic Acid (g/L)	Xylitol (g/L)
0.00	0.09	0.00	3.54	0.21	1.95	
0.00	0.12	0.09	3.48	1.89	2.01	
0.00	0.09	0.11	3.47	2.10	2.08	
0.00	0.08	0,09	3.43	2.16	2.07	
000	0.08	0.18	2.93	1.86	1.85	1.67
0.00	008	0.22	Z89	1.87	1.87	2.21
0.00	006	000	3.48	0.30	2.01	0.00
0.00	0.00	0.34	3.05	Z.88	2.83	2.21

Solids Analysis (% Dry weight)									
Glucose	Xylose	Galactose	Arabinose	Mannose	Klason Lignin	Acid Soluble Lignin	Total Ash	Total Solids	Insol Solids
34.04	24.42	1.30	7.30	0.25	13.78	7.77	0.92	15.65	16.69
12.81	ZZ.0T	3.81	7.41	0.24	19.65	9.95	1.95	17.6	7.23

Byproducts						
Mannose (g/L)	Total Mannose (g/L)	Succinic Acid (g/L)	Lactic Acid (g/L)	Glycerol (g/L)	Acetic Acid (g/L)	Xylitol (g/L)
0.00	0.31	0.01	Z.86	0.15	1.06	
000	0.04	0.0d	2.75	1.00	0.88	
0.00	0.0d	0.06	2.64	0.97	0,93	
0.00	0.0d	0.00	2.64	1.01	0.98	
0.00	0.04	0.11	2.10	0.85	0.90	1.74
0.00	0.0d	0.29	2.44	1.03	1.16	1.90
000	0.00	0.01	Z.86	0.15	I.06	0.00
0.00	000	0.63	1.87	1.11	1.16	T.90

Solids Analysis (% Dry weight)									
Glucose	Xylose	Galactose	Arabinose	Mannose	Klason Lignin	Acid Soluble Lignin	Total Ash	Total Solids	Insol Solids
36.00	21.65	1.42	7.63	0.29	13.02	8.05	1.04	14.19	4.66
10.65	15.79	2.77	4.53	2.43	20.12	10.83	1.54	11.75	2.51